the presynaptic mechanisms that control the loading of synaptic vesicles with transmitter.

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The Great Escape of Glutamate from the Depth of Presynaptic Invaginations

The basal pole of a cone photoreceptor is in close contact with hundreds of bipolar cell dendrites. The function and properties of these unconventional junctions are a long-standing mystery. In this issue of *Neuron*, DeVries and colleagues provide compelling evidence that glutamate release from a single quanta can diffuse to distant AMPA/KA receptors on these basal junctions to generate slow mEPSCs.

How far can glutamate travel from the point of exocytosis at excitatory synapses? This has been a critical question that has been under intense debate for several decades among synaptic physiologists. Some claim that high concentrations of transmitter are confined to a small domain within the postsynaptic density (PSD) area (Franks et al., 2003), and some show strong evidence that glutamate can spill over from the synaptic cleft to activate low-affinity receptors located on nearby synapses (DiGregorio et al., 2002). To operate independently from their neighbors, it would be beneficial for synapses if the released glutamate were confined to an area covering only the directly opposing PSD. Such confinement should maximize the information capacities of a neural circuit. However, it may be necessary to allow for spillover, at least in certain high-intensity activity conditions, to activate perisynaptic receptors, some of which are required for the induction of synaptic plasticity. Thus, spillover may increase the bandwidth of information transfer. Spillover, even to neighboring synapses, may also prove to be beneficial if such spillover causes a low level of activation of receptors at these synapses that could trigger their depression. Such depression at the neighboring synapses could result in a center-surround arrangement that may enhance synaptic specificity (Diamond, 2002). Part of the uncertainty over whether spillover occurs or not stems from the difficulty of measurements of diffusion in the tortuous extracellular space (Thorne and Nicholson, 2006) and from ignorance of the two-dimensional distribution and density of receptors and transporters on the plasma membrane (Tanaka et al., 2005).

In the retina, this question is perhaps even more important and vexing. Cone photoreceptors form the first synapse of the pathway that is responsible for daylight and color vision. To encode visual stimuli over this large dynamic range, the presynaptic terminal of cones forms what is probably the most complex synapse of the vertebrate brain (Figure 1; Haverkamp et al., 2000). The cone terminal forms deep invaginations and also several specialized electron-dense structures called "synaptic ribbons" that lie at the ridge of such invaginations. Many synaptic vesicles cluster around each ribbon, and it is here that most of the exocytosis probably occurs. Horizontal cells send their dendrites deep into the invaginations and end laterally to the ribbon ridge, and the dendrites of depolarizing (On-center) bipolar cells lie centrally within the invaginations, although somewhat away from the synaptic ridge. This slight distance should not be a huge problem, since On bipolar cells express high-affinity mGluR6 receptors, which can react sufficiently even if only a trickle of glutamate spills over. At the basal pole of the cone terminal, several hundred hyperpolarizing (Off-center) bipolar cell dendrites make close contacts, as if poised for a light drizzle of glutamate to escape from the dense thicket of processes in the presynaptic invagination. What is the meaning of this seemingly baroque synaptic achitecture?

Since horizontal cells and On bipolar cells occupy the invaginations, the Off bipolar cells face a potential problem, since they can only contact the photoreceptors at the base of the terminal, and the shortest distance from their dendrites to the ribbon structure within the invaginations can be hundreds of nanometers. Furthermore, cells forming the invaginating synapses express glutamate transporters, and it has been recently suggested that rod photoreceptor terminals may express a surprisingly high density of these transporters (Hasegawa et al., 2006). How can glutamate travel hundreds of nanometers of extracellular space, eluding capture from glutamate transporters, and still reach the dendrites of Off bipolar cells at a concentration sufficient to activate low-affinity AMPA/KA receptors on these cells? DeVries et al. (2006) unequivocally show that this seemingly very difficult task can be done with the release of only a single vesicle.

DeVries et al. (2006) use elegant paired recording techniques from b3/b7 Off bipolar cells and horizontal cells and show coincident occurrence of spontaneous

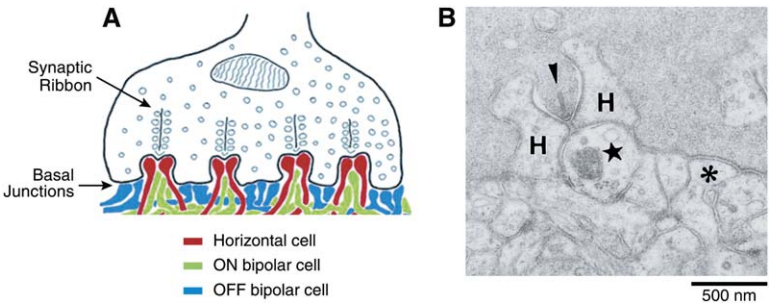

Figure 1. Complex Structure of the Cone Photoreceptor Synapse

(A) This classical schematic drawing illustrates Off bipolar dendrites only at basal contacts; however, DeVries et al. (2006) suggest that a subset of Off bipolar cells sends dendrites into the invaginations to sample high and transient concentrations of glutamate. Other Off bipolar cells make basal contacts and detect smoothed flow of low concentrations of glutamate.

(B) Electron micrograph of a vertical section through the synaptic complex of a cone pedicle base of the Macaque monkey retina. A presynaptic ribbon (arrowhead), two lateral horizontal cell processes ("H"), and one invaginating On bipolar cell dendrite (star) constitute an invaginating synapse. A basal (flat) contact of an Off bipolar dendrite is indicated by an asterisk. Note the relatively long distance of the basal contact from the ribbon structure. Reproduced from Haverkamp et al. (2000), with permission.

responses in the two cells. This suggests that the two cells share input from the same presynaptic release sites. They also found these coincident responses in b3/b7 bipolar cells to be significantly slower than those in horizontal cells. This difference could potentially be due to the difference in the kinetic properties of the respective postsynaptic receptors. However, outside-out patch experiments revealed that such slow responses found in b3/b7 bipolar cells could only be induced by low concentration transients of glutamate. In addition, they found that responses from another class of Off bipolar cell, the b2 bipolar cell, has similar kinetics as horizontal cells, and thus they conclude that the dendrites of the b2 bipolar cell must be "functionally" invaginating and sampling similarly high concentrations of glutamate transients as those sensed by the horizontal cells.

Computer simulations also suggested that glutamate concentrations reaching b3/b7 bipolar cell dendrites must be low (<100 μM). Such low concentration of glutamate should provide a very low open probability for AMPA/KA receptors (<0.05) since the AMPA/KA receptors expressed on these cells have relatively low affinity for glutamate ($EC_{50} \approx 350$ μM). However, sizable quantal responses are recorded from these cells (>2 pA). This suggests that high densities of AMPA/KA receptors must be expressed at the tip of their dendrites. It would be of great interest to be able to measure the absolute density and distribution of the AMPA/KA receptors relative to the ribbon on these bipolar cells (Haverkamp et al., 2001). Along with the data presented by DeVries et al. (2006), this could provide the long sought parameter, the glutamate diffusion coefficient in the extracellular space (Rao-Mirotznik et al., 1998).

Interestingly, at the molecular layer of the cerebellum, similar techniques have resulted in completely opposite conclusions. Matsui et al. (2005) recorded from two "postsynaptic" cells, the Purkinje cell and the surrounding Bergmann glial cell, sharing input from the same single presynaptic climbing fiber. At this powerful cerebellar synapse, they did not find coincident occurrence of quantal responses in the two cells, and they also found from the receptor kinetic modeling that the glutamate concentration transients reaching the Bergmann glia is as high as that in the synaptic cleft (Matsui et al., 2005). From these results, they conclude that quantal responses in Bergmann glial cells are mediated by ectopic exocytosis of synaptic vesicles from presynaptic membrane directly facing glial cells and that spillover from the release of a single vesicle in the synaptic cleft does not result in sufficient concentrations of glutamate to activate the low-affinity AMPA receptors expressed on these glial cells.

The work of DeVries et al. (2006) thus provides an interesting contrast to these previous works suggesting that the degree of spillover may depend on synaptic architecture. Several mechanisms remain to be studied for how to facilitate or hinder spillover at different CNS synapses (Renden et al., 2005), such as differences in the extracellular geometry, differences in the diffusion properties, differences in the packaging amount of glutamate inside a vesicle, differences in the location and density of glutamate transporters, and the occurrence of spontaneous multivesicular release at ribbon synapses. The work of DeVries et al. (2006) clearly shows that ectopic release is not mediating the responses of spontaneous events, or small evoked responses; however, it would be of interest whether ectopic release directly facing the basal contact could occur following strong or prolonged excitation of the cone photoreceptors.

Concerning the functional significance for spillover, if different shades of glutamate are supplied by the cone photoreceptor terminal complex (Haverkamp et al., 2000), it would seem to provide an ideal environment, where the postsynaptic cells could control the sensitivity and the temporal frequency of their responses by allocating their processes at adequate locations relative to the ribbons. It would also be of great interest to see whether the tips of the dendrites of Off bipolar cells can be actively controlled, during adaptation for example, as has been suggested to occur for horizontal cell spinules in fish retina (Wagner and Djamgoz, 1993).

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